CLINICAL STUDY

HereditY and lifestyle in the determination of between-subject variation in thyroid hormone levels in euthyroid men

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Abstract

Objective: Variation in thyroid hormone (TH) concentrations between subjects is greater than in a single subject over a prolonged period of time, suggesting an individual set point for thyroid function. We have previously shown that TH levels within normal range are associated with clinical indices such as bone mass, BMI, and heart rate. The aim of this study on young men was therefore to gain insight into the determinants of variation in TH levels among healthy subjects.

Methods: Healthy male siblings ($n=941$, 25–45 years) were recruited in a cross-sectional, population-based study; a history or treatment of thyroid disease and thyroid auto-immunity were exclusion criteria. A complete assessment of TH status was performed (TSH, free thyroxine (FT₄), free triiodothyronine (FT₃), thyroperoxidase, and thyroglobulin antibodies, reverse T₃ (rT₃), thyroid-binding globulin (TBG), and urinary iodine levels). Genotyping was performed by TaqMan and KASP (KBiosciences) genotyping assays.

Results: (F)T₄, rT₃, and TBG had heritability estimates between 80 and 90%. Estimates were lower for (F)T₃ (60%) and lowest for TSH (49%).

Significant associations were observed between different single-nucleotide polymorphisms (SNPs) in the thyroid pathway and TSH, FT₄, ratio FT₃:FT₄, and rT₃. Nevertheless, these SNPs only explain a limited part of the hereditY. As to age and lifestyle-related factors, (F)T₃ was negatively related to age and education level, positively to smoking and BMI (all $P<0.0001$) but not substantially to urinary iodine concentrations. Smoking was also negatively related to TSH and positively to FT₄.

Conclusion: Both genetic and lifestyle-related factors play a role in determining between-subject variation in TH levels in euthyroid young men, although genetic factors seem most important.

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Introduction

In healthy subjects, thyroid-stimulating hormone (TSH) and thyroid hormone (TH) concentrations can show substantial differences between individuals (inter-individual variation), whereas variability is much less in the same individual over a prolonged period of time (intra-individual variation). This suggests an individual set point for pituitary–thyroid axis function (1). We and others have shown that this between-subject variation in TH levels, although within the normal range, is nevertheless associated with a number of clinical parameters such as bone mass, BMI, metabolic indices, and heart rate (2, 3, 4, 5). The physiological basis of the set point of this axis is poorly understood (6). Twin studies demonstrate a moderately strong genetic influence (7, 8, 9, 10, 11). In recent years, several of the genes involved in this regulation have been identified, with common genetic variation in phosphodiesterase 8B (PDE8B), TSH receptor (TSHR), deiodinase 1 (DIO1), and capping protein muscle Z-line beta (CAPZB)

being associated with circulating THs (12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22). Besides, some of these single-nucleotide polymorphisms (SNPs) in the TH pathway have also been associated with clinical characteristics such as lean mass, bone density, hypertension, and insulin resistance (23, 24, 25, 26, 27, 28). Nevertheless, most of the heritability remains unexplained, suggesting that many more regulatory genes remain to be identified and/or that rare genetic variants may be important (6).

Besides genetic factors, age and lifestyle-related factors can also have an important influence on TH levels. Aging (29, 30), smoking (31, 32), body composition (3, 33), and differences in iodine intake (34, 35, 36) were all reported to influence TSH and TH concentrations, although not all studies agree on the magnitude of the effects.

Few studies have investigated genetic together with lifestyle-related determinant factors of thyroid function (8, 11). This study therefore investigates the contribution of genetics, i.e. both heritability in general as

well as the effects of specific SNPs in the TH pathway, together with lifestyle-related factors in the determination of between-subject variation in TH levels in a well-characterized population of 941 healthy euthyroid brothers between 25 and 45 years old.

Subjects and methods

Study design and population

Participants were recruited from the population registries of the semi-rural to suburban communities around Ghent, Belgium. Although the initial focus of this study in healthy young men was on the determinants of sex steroid levels and peak bone mass, its scope has been extended to include the determinants and clinical correlates of TH levels. Inclusion criteria and study design were described previously (37, 38). Men aged 25–45 years old were contacted by direct mailing, briefly describing the study purpose and were asked if they had a brother within the same age range also willing to participate. Finally, a sample of 1114 men agreed to participate. After exclusions, 1001 men were included in the study: 435 brother pairs, 25 families with three brothers and two families with four brothers. A total of 92 men were included as single participants, when their brother could not participate in the study. All analyses were done taking into account the family structure. The maximal age difference within brother pairs was arbitrarily set at 12 years. All participants were in good health and completed questionnaires about previous illnesses, smoking (never/former/present smoker), food intake (calcium intake), education (years of education), physical activity, and medication use. Exclusion criteria were defined as illnesses or medication use affecting body composition, hormones or bone metabolism, current or prolonged (> 3 months) use of glucocorticosteroids, anti-androgens, vitamin D supplements, insulin, thyroxine (T_4), previous or current use of anti-epileptic drugs, hypogonadism, hyperthyroidism, cystic fibrosis, malabsorption or eating disorders, disorders of collagen metabolism or bone development, chronic renal failure, alcohol abuse, and autoimmune rheumatoid disease. All subjects were tested for the presence of thyroid auto-antibodies and those with serum levels above the clinical cut-off (thyroperoxidase antibody (TPOAb) > 35 U/l or thyroglobulin antibody (TgAb) > 115 U/l) were additionally excluded (60 persons or 5.3% of our population), leaving 941 subjects. The study protocol was approved by the Ethical Committee of the Ghent University Hospital and written informed consent was obtained from all participants. Smoking habits were registered as current or previous smoking.

Body weight was measured in light indoor clothing without shoes. Standing height was measured using

a wall-mounted Harpenden Stadiometer (Holtain Ltd., Crymch, UK).

Biochemical determinations

Venous blood and urine samples were obtained between 0800 and 1000 h after overnight fasting. All serum samples were stored at -80°C until batch analysis. Thyroid tests included TSH, free T_4 (FT₄), free triiodothyronine (FT₃), total T_3 (TT₃), TT₄, reverse T_3 (rT₃), and thyroid-binding globulin (TBG) as well as TPOAbs and TgAbs. All measurements with the exception of TBG and rT₃ were performed using immuno-electrochemiluminescence (Roche reagents) on Modular E or Cobas 411 (Roche Diagnostics GmbH). TBG and rT₃ were measured by a RIA (DIAsource ImmunoAssays S.A., Nivelles, Belgium). A commercial RIA was used to determine serum levels of estradiol (E_2 ; Clinical assay, DiaSorin s.r.l., Saluggia, Italy).

Urine samples were stored at -18°C until assayed. Urinary iodine was determined using inductively coupled plasma mass spectrometry on a PerkinElmer Elan DRC-e equipped with a standard cross-flow nebulizer and a dynamic reaction cell (PerkinElmer). Results are quantitatively reported if the concentrations exceed the limit of quantification, i.e. 4 µg/l; samples above the upper limit of quantification (500 µg/l) were diluted before reanalysis. The laboratory participates in the external quality control program Quebec Multi-Element External Quality Assessment Scheme (Canada).

Creatinine in urine was determined by the kinetic Jaffé-method on a Cobas 411 (Roche Diagnostics GmbH). The iodine (µg):creatinine (g) ratio was calculated by dividing urinary iodine by urinary creatinine and multiplying the result by 100. As we studied only Caucasian men of a particular age category (25–45 years), additional adjustment for sex, race, or age was not necessary (39). The intra- and interassay coefficients of variation were below 10% for all measurements.

Genotyping

The SNPs in DIO1 (rs11206244 and rs2235544), DIO2 (rs225014), and TRHR (rs7832552) were determined by TaqMan Pre-Designed SNP Genotyping assays (Applied Biosystems), which were run on the StepOne System (Applied Biosystems). Genotyping was successful in over 98% of the four SNPs, with error rates of ~0.5%.

All other SNPs were genotyped by KBiosciences using KASPar Technology (LGC, Middlesex, UK). KASP is a competitive allele-specific PCR, incorporating a fluorescent resonance energy transfer quencher cassette. Genotyping was successful for over 97% of the sample across the five SNPs, with error rates of ~0.5%. None of the SNPs deviated significantly from Hardy–Weinberg equilibrium.

Table 1 Population characteristics and descriptive parameters of studied variables. General characteristics, thyroid hormone parameters and parameters of iodine intake of all study participants ($n=941$). Total population consisted of 1001 subjects. Subjects with positive thyroid autoimmunity were excluded (60 subjects or 5%), leaving 941 subjects. Variables are given as mean $\pm$ s.d.; when there was a non-Gaussian distribution, data were presented as medians (1st–3rd quartiles). Conversion factor for FT₃ from pg/dl to pmol/l, for TT₃ from ng/dl to nmol/l, and for rT₃ from ng/dl to nmol/l is 0.0154; conversion factor for FT₄ from ng/dl to pmol/l and for TT₄ from μ g/dl to nmol/l is 12.87; and conversion factor for TBG from mg/dl to mg/l is 0.1.

Parameters	Mean $\pm$ s.d./median (IQR)
Age (years)	34 (30–39)
Height (cm)	179.6 $\pm$ 6.5
Weight (kg)	79 (73–87.5)
BMI (kg/m ²)	24.6 (22.7–26.9)
Smokers	24%
TSH (mU/l)	1.6 (1.2–2.1)
FT ₄ (ng/dl)	1.4 $\pm$ 0.2
FT ₃ (pg/dl)	383 $\pm$ 40
TT ₄ (μ g/dl)	8.5 $\pm$ 1.5
TT ₃ (ng/dl)	129 $\pm$ 20
TBG (mg/dl)	1.8 $\pm$ 0.3
rT ₃ (ng/dl)	0.29 $\pm$ 0.07
Urinary iodine (μ g/l)	74 (54–99)
Urinary creatinine (mg/dl)	167 (126–213)
Iodine/creatinine (μ g/g)	0.05 (0.04–0.06)

Statistical analysis

Descriptive parameters are expressed as mean $\pm$ s.d. or median (1st–3rd quartiles) when criteria for normality were not fulfilled (Kolmogorov–Smirnov) and dependent variables (hormone concentrations) were log-transformed in subsequent linear models. Linear mixed-effects modeling with random intercepts and a simple residual correlation structure for random effects were used to evaluate cross-sectional relationships in our study population, taking the interdependence of measurements within families into account. Parameters of fixed effects were estimated via restricted maximum likelihood estimation and reported as standardized estimates of effect size (β) with their respective s.e.m. Estimates for the SNPs were calculated using an additive model. Significance levels for associations were set at P values ≤ 0.05 . P values in figures result from ANOVA. Statistical analyses were performed using Spotfire S+ 8.1 (Insightful, Seattle, WA, USA) and MedCalc (Mariakerke, Belgium).

Taking advantage of the family structure of the dataset, the polygenic program in SOLAR 4.0 (Southwest Foundation for Biomedical Research, San Antonio, TX, USA) was used to estimate the (shared) heritability of TH concentrations (40).

Results

Men with positive TPO or TG antibodies were excluded from further analyses, as stated in the ‘Subjects and

methods’ section. TH concentrations for men without thyroid auto-immunity are given in Table 1, together with the other descriptive parameters of the population.

Genetic determinants of thyroid function

Heritability Heritability estimates for TH concentrations are listed in Table 2. Estimates for FT₄, TT₄, rT₃, and TBG are all high and in the same range between 80 and 90%. The estimates for FT₃ and TT₃ are considerably lower (60%). The lowest heritability estimate is observed for TSH (49%).

SNPs in the TH pathway Associations between the presence of SNPs in the TH pathway and concentrations of TSH, FT₄, FT₃, TT₄, TT₃, rT₃, and the ratio FT₃:FT₄ are given in Table 3; Figs 1, 2 and 3. A total of nine SNPs were determined. (Log)TSH is highly, significantly, positively associated with the presence of rs4704397 in PDE8B (explaining 1.5% of variation in an unadjusted model), and negatively with the presence of rs13063628 in THRB, although the latter association is less significant (explaining 0.5% of the variation). Two SNPs in the TSHR (rs10149689 and rs12050077) are negatively associated with FT₄ concentrations (both explaining 1% of the variation in an unadjusted model) (Fig. 2). The other SNP in TSHR, rs1991517, does not show associations with TH concentrations. Significant associations with FT₄ concentrations are observed for two SNPs in DIO1; a positive association for rs11206244 and a negative association for rs2235544 (both explaining 0.5% of variation). In agreement with this negative association with FT₄, this last SNP (rs2235544) also shows a negative association with TT₄-levels ($\beta = -0.10 \pm 0.05$, $P = 0.03$). In addition, these SNPs are positively associated with the ratio FT₃:FT₄, explaining 1% of variation (Fig. 3). Finally, several associations with rT₃-concentrations are observed: strong associations with the two SNPs in DIO1, rs11206244 (positive) and rs2235544 (negative) (Fig. 3), and borderline-significant associations for the two SNPs in TSHR, rs10159689 and rs12050077 (negative), and for the SNP in DIO2 (negative).

As we replicated previously studied SNPs, we did not adjust significance levels for multiple testing. Nevertheless, it can be mentioned that adjustment

Table 2 Heritability estimates (unadjusted) $\pm$ s.d.

Thyroid parameters	Heritability (h^2)	P value
Log(TSH)	0.49 $\pm$ 0.09	3.8×10^{-9}
FT ₄	0.89 $\pm$ 0.08	3.6×10^{-25}
TT ₄	0.80 $\pm$ 0.08	1.1×10^{-20}
FT ₃	0.60 $\pm$ 0.09	1.5×10^{-11}
TT ₃	0.58 $\pm$ 0.09	3.7×10^{-11}
TBG	0.82 $\pm$ 0.08	7.1×10^{-22}
rT ₃	0.88 $\pm$ 0.08	5.5×10^{-25}

Table 3 Associations between SNPs in the thyroid hormone (TH) pathway and TH parameters. Results from linear mixed effect modeling, with the different TH parameters as dependents and the polymorphisms as independents. Betas are standardized and reported together with their s.d. Associations were unadjusted. R^2 is given for the significant associations. When there was no significant association, NA was reported. Statistically significant ($P \leq 0.05$) values are in boldface.

Gene	Polymorphism	Minor allele	Log(TSH)		FT ₄		FT ₃		FT ₃ /FT ₄		rT ₃		R^2 (%)
			β (s.e.m.)	P	β (s.e.m.)	P	β (s.e.m.)	P	β (s.e.m.)	P	β (s.e.m.)	P	
PDE8B	rs4704397	A	0.18 (0.05)	0.0002	-0.08 (0.05)	0.1	-0.02 (0.05)	0.7	0.07 (0.05)	0.15	-0.08 (0.05)	0.08	1.5
TRHR	rs7832552	T	-0.05 (0.05)	0.3	-0.04 (0.05)	0.5	0.01 (0.05)	0.9	0.04 (0.05)	0.5	-0.06 (0.05)	0.2	NA
TSHR	rs10149689	G	0.07 (0.05)	0.1	-0.13 (0.04)	0.009	-0.04 (0.05)	0.4	0.08 (0.05)	0.08	-0.10 (0.05)	0.038	1
	rs12050077	A	0.07 (0.05)	0.2	-0.12 (0.05)	0.01	-0.05 (0.05)	0.3	0.08 (0.05)	0.1	-0.14 (0.05)	0.044	1
	rs1991517	G	-0.10 (0.08)	0.2	-0.04 (0.08)	0.6	0.2 (0.08)	0.9	0.07 (0.08)	0.4	-0.08 (0.08)	0.3	NA
DIO1	rs11206244	T	0.04 (0.05)	0.4	0.11 (0.05)	0.03	-0.07 (0.05)	0.2	-0.16 (0.05)	0.002	0.25 (0.05)	<0.0001	1
	rs2235544	C	-0.09 (0.05)	0.06	-0.11 (0.05)	0.02	0.06 (0.05)	0.2	0.16 (0.05)	0.0006	-0.25 (0.05)	<0.0001	1
DIO2	rs225014	G	0.02 (0.05)	0.8	-0.08 (0.05)	0.09	-0.03 (0.05)	0.5	0.07 (0.05)	0.14	-0.11 (0.05)	0.03	NA
THRB	rs13063628	A	-0.15 (0.07)	0.03	0.01 (0.07)	0.9	-0.02 (0.07)	0.8	-0.01 (0.07)	0.9	-0.03 (0.07)	0.6	NA

PDE8B, phosphodiesterase 8B; TRHR, thyrotropin-releasing hormone receptor; TSHR, thyroid-stimulating hormone receptor; DIO1, deiodinase 1; THRB, thyroid hormone receptor β ; NA, not applicable.

for multiple comparisons according to Bonferroni would require a P value of 0.006 (0.05 divided by 9) for significance. According to this criterion, only the associations of the SNP in PDE8B with TSH and of rT₃ and the FT₃:FT₄ ratio with the SNPs in DIO1 are significant.

Age and lifestyle-related determinants of thyroid function

Associations between parameters of thyroid function and age and different lifestyle-related covariates in unadjusted models are listed in Table 4. Even though the age range in this cohort is rather narrow and only young to middle-aged men (25–45 years) are considered, age is negatively associated to all parameters of thyroid function and explains between 2 and 3.5% of the variation in TH parameters.

Smoking is negatively associated with TSH and positively with all other thyroid parameters. It explains 1% of the variation in TSH and (F)T₄ and 3% of the variation in (F)T₃. Additional adjustment for TBG or E₂ does not change the observed associations between smoking and thyroid parameters (data not shown).

BMI is positively associated with FT₃, TT₃, TBG, and rT₃, explaining about 2% of the variation in these parameters.

The median level of iodine in urine is 0.074 $\mu\text{g}/\text{ml}$ (0.054–0.099), indicating a mild iodine deficiency in this cohort. Spot urinary iodine concentrations are not related to any parameter of TH status, except for a negative relation with rT₃ concentrations (explaining 0.5% of the variation). The urinary iodine:creatinine ratio, however, is significantly negatively associated with FT₄ and rT₃ levels (explaining 0.5 and 2% of the variation respectively). There is also a non-significant trend toward a negative association with TT₄. Besides, FT₄, FT₃, and rT₃ levels are significantly different between subjects from different quartiles of urinary

Figure 1 TSH-levels according to genotype for the rs4704397 polymorphism in PDE8B. P value results from ANOVA.

Figure 2 TSH and FT₄-levels (ANOVA) according to genotype of SNPs in TSHR (rs10149689 and rs12050077). For rs10149689, genotype 0 refers to AA, 1 to AG, and 2 to GG. For rs12050077, genotype 0 refers to GG, 1 to GA, and 2 to AA.

iodine:creatinine ratio, with a trend for lower concentrations with a higher quartile (Fig. 4). Furthermore, the ratio is positively associated with calcium intake in this population ($\beta = 0.12 \pm 0.03$, $P < 0.0001$), indicating a positive association between intake of dairy products and urinary iodine. Urinary iodine levels or serum TSH and THs were not related to the season in which the examinations took place.

Education level is negatively associated to (F)T₃, the ratio FT₃:FT₄ and TBG, as well as to BMI ($\beta = -0.10 \pm 0.03$, $P = 0.002$) and smoking ($\beta = -0.30 \pm 0.08$, $P < 0.0001$). It explains 3% of the variation in (F)T₃ and the ratio FT₃:FT₄. Nevertheless, the associations between education and thyroid parameters remain significant after adjustment for smoking and BMI. Besides, a higher level of physical activity during the job is positively associated to (F)T₃ ($\beta = 0.18$, $P < 0.0001$) and negatively to (F)T₄ ($\beta = -0.13$, $P < 0.0001$), whereas the exertion of sport in the subjects' free time is negatively related to (F)T₃ ($\beta = -0.15$, $P < 0.0001$) and (F)T₄ ($\beta = -0.09$, $P = 0.001$). Sport exertion in free time is also negatively associated with BMI, whereas a physical active job does not influence BMI (data not shown). When the different lifestyle-related determinants are considered together in a multiple regression model, the observed associations from the unadjusted models remain largely significant (data not shown).

Discussion

In this study, we have investigated genetic and lifestyle-related determinants of TH function in a well-characterized population of 941 healthy, euthyroid young to middle-aged brothers.

Our observations on heritability, with the highest estimates for (F)T₄, TBG, and rT₃, lower estimates for (F)T₃, and the lowest estimate for TSH, are partially in agreement with earlier findings. Reported estimates range from 32 to 65% for TSH, from 37 to 65% for FT₄, and from 23 to 67% (7, 10, 11). The observation of the lowest estimate for TSH is in agreement with observations from Samollow *et al.* (11). Nevertheless, other studies found relatively higher estimates for TSH than for (free) THs (41), or estimates lying within the same range as for free THs (7). Some authors hypothesize that variation in serum TSH concentrations is under a stronger genetic influence and lesser environmental influence than (F)T₄ and (F)T₃ (10). We could not confirm this hypothesis, although we did observe lower heritability estimates for (F)T₃ than for (F)T₄, which suggests a stronger influence of lifestyle and other environmental factors on T₃ levels compared with T₄. The generally somewhat higher heritability estimates in this study might result from the fact that a sib-pair design is less able to discriminate between shared environmental and genetic effects than twin studies, resulting in an overestimation of heritability, but also from the fact that we studied a well-characterized rather homogenous population.

In view of the high heritability estimates for most thyroid parameters, which indicate that a substantial part of the between-subject variation has a genetic basis, we assessed in our cohort of healthy euthyroid young men the possible contribution of a number of

Figure 3 FT₄, rT₃, and ratio FT₃:FT₄ according to genotype of SNPs in DIO1 (rs11206244 and rs2235544). P values result from ANOVA. For rs11206244, genotype 0 refers to CC, 1 to CT, and 2 to TT; for rs2235544, genotype 0 refers to AA, 1 to AC, and 2 to CC.

Table 4 Associations between age and possible lifestyle-related determinants and the different thyroid hormone parameters. Results from linear mixed-effect modeling, with the different thyroid hormones used as dependents and the lifestyle-related determinants as independents. Standardized estimates are reported. Associations were unadjusted. R^2 is given for the significant associations. When there was no significant association, NA was reported. Statistically significant ($P \leq 0.05$) values are in boldface.

Determinant	Log(TSH)	FT ₄	TT ₄	FT ₃	TT ₃	FT ₃ /FT ₄	TBG	rT ₃	R ² (%)
Age (years)	-0.13 ± 0.03 P=0.0003	-0.18 ± 0.03 P<0.0001	-0.11 ± 0.03 P=0.002	-0.15 ± 0.03 P<0.0001	-0.05 ± 0.03 P=0.12	0.06 ± 0.03 P=0.07	-0.02 ± 0.03 P=0.5	-0.24 ± 0.03 P<0.0001	2–3.5
Smoking	-0.23 ± 0.08 P=0.003	0.24 ± 0.07 P=0.0009	0.25 ± 0.07 P=0.0006	0.36 ± 0.07 P<0.0001	0.47 ± 0.08 P<0.0001	0.04 ± 0.07 P=0.6	0.19 ± 0.07 P=0.01	0.17 ± 0.07 P=0.02	1–3
BMI (kg/m ²)	-0.04 ± 0.03 P=0.3	-0.06 ± 0.03 P=0.06	0.02 ± 0.03 P=0.6	0.15 ± 0.03 P<0.0001	0.15 ± 0.03 P<0.0001	0.17 ± 0.03 P<0.0001	0.09 ± 0.03 P=0.005	-0.09 ± 0.03 P=0.009	2
Urinary iodine (µg/ml)	-0.04 ± 0.03 P=0.2	-0.03 ± 0.03 P=0.3	-0.03 ± 0.03 P=0.3	-0.01 ± 0.03 P=0.8	-0.02 ± 0.03 P=0.5	0.02 ± 0.03 P=0.5	-0.05 ± 0.03 P=0.1	-0.06 ± 0.03 P=0.04	0.5
Iodine _{urine} /creatinine _{urine} (µg/mg)	-0.02 ± 0.03 P=0.5	-0.08 ± 0.03 P=0.007	-0.05 ± 0.03 P=0.07	-0.04 ± 0.03 P=0.3	-0.03 ± 0.03 P=0.4	0.05 ± 0.03 P=0.1	-0.02 ± 0.03 P=0.5	-0.10 ± 0.03 P=0.0007	0.5–2
Education (years)	0.05 ± 0.03 P=0.1	0.04 ± 0.03 P=0.3	-0.01 ± 0.03 P=0.7	-0.17 ± 0.03 P<0.0001	-0.14 ± 0.03 P<0.0001	-0.16 ± 0.03 P<0.0001	-0.07 ± 0.03 P=0.02	0.05 ± 0.03 P=0.09	3

NA, not applicable.

different SNPs in the genes involved in thyroid regulation and previously reported to be associated with TH levels in other populations. We observe robust significant associations between two SNPs in *DIO1* (in linking disequilibrium, $r^2=0.41$) and both the ratio FT₃:FT₄ and rT₃ levels. There was also a weaker association of (F)T₄ levels with these SNPs. The observed associations for the two SNPs are in opposite directions and in agreement with previous studies, which described a higher deiodinase activity for SNP rs2235544 and a lower deiodinase activity for rs11206244 respectively (15, 22).

We observed a rather unexpected negative association between the presence of rs225014 in *DIO2* and rT₃ levels. As this SNP has been reported to be associated with a lower D2 activity (28, 42), one rather might expect higher than lower rT₃ levels. We do not have a physiological explanation for this observation. Furthermore, the presence of the SNP has been linked to a lower femoral neck bone mineral density, insulin resistance, and hypertension. Nevertheless, none of these studies observed an association between this SNP and circulating THs (23, 24, 28, 42).

We observe no association between the rs7832552 polymorphism in the *TRHR* gene and circulating TH levels or TSH. A genome-wide study has previously identified a significant association between the presence of this SNP and higher lean body mass (25), but to the best of our knowledge, no other study has investigated associations with TH levels so far.

The highly significant association between rs4704397, a SNP in *PDE8B*, and higher TSH levels has previously been observed in several large cohorts (12, 14, 21, 43, 44). We also have recently confirmed this positive association with TSH as well as a negative association with FT₃ and FT₄ levels in another large cohort (45). However, the latter negative relations with free THs could not be confirmed in this study, possibly

Figure 4 FT₃, FT₄, TT₄, and rT₃ levels according to quartiles of the ratio iodine:creatinine. P values result from ANOVA (unadjusted model).

due to the smaller size of the present cohort and ensuing lower power.

At variance with previous reports of associations of SNPs in the TSH-receptor (46, 47), we did not observe any associations between three SNPs in TSHR with TSH levels in our study. Nevertheless, in our cohort, rs10149689 and rs12050077 in TSHR are negatively associated with FT₄-levels, which points toward an effect of these polymorphisms on TSH action, resulting in a decreased thyroid function.

The SNP in *THRB*, rs13063628, has been associated with higher TSH levels in Danish twins, but this could not be confirmed in the Rotterdam study (48). Surprisingly, in our study, we even observe a negative association between the presence of this SNP and TSH levels, although the significance level of the association was rather low.

A SNP in *CAPZB*, rs10917469, has been related to TSH levels as well, but this SNP was not determined in our cohort. However, in agreement with our observations for the other SNPs, this SNP also explained only about 1% of the variation in TSH levels (16).

Obviously, there are numerous candidate genes implicated in the complex determination of circulating TH levels. Here, the identified contributing SNPs each explain only a very small fraction of the genetic determination of between-subject variability, hereby underlining the polygenetic character of the heritability of TH levels. In addition, the discrepancy between the high heritability estimates and low r^2 of studied SNPs might also be caused by gene–environment interactions or even by epigenetic phenomena. Indeed, evidence suggests that epigenetics links genetics and environment in shaping endocrine function. With regard to TH regulation, the expression of the sodium iodide symporter was shown to be regulated by cytosine methylation of its promotor. Besides, epigenetic regulation of *TSHR* has been demonstrated (49).

Beside genetics, we assessed the role in the determination of TH levels of age and lifestyle-related variables, including BMI, smoking, education, sport exertion, and iodine intake. Notwithstanding the rather narrow age range in our study population, we observe inverse associations between increasing age and TSH as well as THs. Previous studies on this topic have not been straightforward: both an increase as well as a decrease in TSH with progressing age has been observed (29, 30, 34, 50). Differences in iodine status of a population might be an explanation for this paradox as well as the thoroughness of exclusion of subjects with thyroid auto-immunity. Our finding of a clear effect of age in a relatively young, healthy population is noteworthy.

Smoking is associated with slightly higher TH levels (both free and total) together with lower TSH levels. A larger influence of smoking on (F)T₃ compared with (F)T₄ levels is observed, as well as a higher R^2 . In our population, smoking is also positively associated with TBG and rT₃. These results are in line with previous

studies from Soldin *et al.* (31) and Asvold *et al.* (32). Proposed mechanisms are: activation of the sympathetic nervous system by nicotine, resulting in a positive effect on TH secretion (51); higher TBG and testosterone levels together with lower E₂ levels in smokers (52); or a direct stimulating effect of smoking on the thyroid gland (51). Here, as the observed associations between smoking and thyroid parameters remained significant after adjustment for TBG and E₂, we have no reason to believe that indirect effects through sex steroids are the mediators for the observed associations.

We discussed associations between BMI and other parameters of body composition with TH levels in a previous paper (3). Briefly, we have observed that a less favorable body composition (with a higher BMI, higher fat, and lower muscular mass) is associated with higher T₃ levels and an increased FT₃:FT₄ ratio, possibly mediated by higher leptin levels. In keeping with these findings, we report here an inverse relation between years of education and T₃ levels, which might be related to a healthier lifestyle (with a lower caloric intake and lower smoking rate) in subjects with a higher education. Besides, we observe an inverse relation between free THs and physical activity, which might be related to our findings on body composition and THs.

Median fasting morning urinary iodine levels in our cohort are in agreement with acknowledged mild iodine deficiency in Belgium (53, 54). Uncorrected urinary iodine levels were not associated to thyroid parameters in this cohort, in line with a previous large study (NHANES III) (35). However, the iodine:creatinine ratio was negatively associated with FT₄ and rT₃ in our study. Studies longitudinally evaluating subjects after iodine fortification have shown a rather surprising positive association between urinary iodine excretion and TSH levels (34, 50). In iodine-sufficient populations, findings suggest a positive linear relation between iodine levels and TSH (36), whereas in subjects with urinary iodine excretion below 50 µg/24 h (moderate iodine deficiency), a negative correlation between urinary iodine and TSH was observed (55). Nevertheless, on a population basis, the impact of iodine on TH levels seems limited in comparison with other known covariates (35). Iodine intake is related to the consumption of dairy products in our population, in agreement with a previous study suggesting that milk is the main source of iodine in Belgium (54). However, we do not observe seasonal variation in urinary iodine or TH levels, in contrast with a recent study of Moreno-Reyes *et al.* (53).

The major strength of our study is the extensive phenotypic characterization of the population, together with information on both genetic as well as lifestyle-related determinants. A limitation is the absence of thyroid ultrasound measurements, not allowing us to give information on thyroid size or morphology. Besides, due to this absence of thyroid ultrasound measurements, it cannot be excluded that a number of

subjects without thyroid antibodies still suffered from a Hashimoto's thyroiditis, because up to 15% of the subjects with Hashimoto lack thyroid antibodies. Another limitation is that we only have one sample of urinary iodine from each subject. As there is a considerable day-to-day variation in urinary iodine excretion, this might have biased our results.

In conclusion, in this study we investigated genetic together with lifestyle-related determinants of thyroid levels in a population of healthy, euthyroid young men. We have shown moderate to high heritability estimates, with identified contributing gene polymorphisms each explaining only a small part of between-subject variability. Genetics, age, and lifestyle-related factors, such as smoking, education level, and body composition, are significant codeterminants of TH levels, whereas iodine intake was found to only minimally influence circulating TH. Noteworthy is that (F)T₃ appears to be influenced to a larger extent by environmental factors than the other studied thyroid function indices.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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